Programa de Doctorado en Bioingeniería

Funciones morfogenéticas de los genes *ANU1*, *ANU4*, *ANU9*, *ANU12*, *SCA1*, *SCA5*, *ICU11* y *CP2* de Arabidopsis

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HAGO CONSTAR:

Que el presente trabajo ha sido realizado bajo mi dirección y recoge fielmente la labor desarrollada por el Licenciado Eduardo Mateo Bonmatí para optar al grado de Doctor. Las investigaciones reflejadas en esta Tesis se han desarrollado íntegramente en la Unidad de Genética del Instituto de Bioingeniería de la Universidad Miguel Hernández de Elche.

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III.- SUMMARY

We studied Arabidopsis leaf mutants previously isolated in the laboratory of José Luis Micol, where these mutants had already been assigned to the Angulata (Anu), Scabra (Sca) and Incurvata (Icu) phenotypic classes. The *anu* mutants are smaller than the wild type, with pale-green leaves of prominent marginal teeth. Leaves from the *sca* mutants show variegation, dentate margins and irregular lamina surfaces. The *icu* mutants exhibit curly leaves.

Here, we contribute to the characterization of the *anu1-1*, *anu4*, *anu9-1*, *anu12*, *sca1*, *sca5* and *icu11-1* mutants. The marginal teeth and depigmentation shared by *anu* and *sca* leaves indicate defective photosynthesis and suggest that these mutants might be useful for the study of the relationship between chloroplast biogenesis and whole leaf organogenesis. Leaf hyponasty in the *icu* mutants indicates altered dorsoventrality and suggests their usefulness for the study of dorsal (adaxial) and ventral (abaxial) tissue growth, and/or their coordination.

All the mutations studied in this work had already been mapped by linkage analysis to molecular markers, which delimited candidate intervals ranging from 30 to 750 kb. We studied the mutations within these intervals by massive sequencing. This strategy allowed us to identify the *ANU1*, *ANU4*, *ANU9*, *ANU12*, *SCA1* and *SCA5* genes. We confirmed the correct identification of these genes through allelism tests and complementation mediated by transgenes carrying the corresponding wild-type alleles.

ANU1 was found to be At1g21650, already named *SECRETIONA2* (*SECA2*) by previous authors, which encodes a protein with ATPase activity, localized at the stroma of the chloroplasts, and involved in the Sec system, which imports proteins to the thylakoids. *ANU4* is At1g02280, which encodes the TRANSLOCON AT THE OUTER ENVELOPE MEMBRANE OF CHLOROPLASTS 33 (TOC33), one of the components of the TOC complex, which imports proteins from the cytoplasm. *ANU9* is At5g14100, which encodes NON-INTRINSIC ABC PROTEIN 14 (NAP14), another transporter in the chloroplast envelope. *ANU12* is At1g49970, which encodes CASEINOLYTIC PROTEASE RING1 (ClpR1), a component of the Clp complex for chloroplast protein degradation. We used the *anu1*, *anu4*, *anu9* and *anu12* mutants to test the validity of massive sequencing to identify causal genes for phenotypes of interest. Since these four genes have been characterized to different extents by previous authors, we decided not to continue their study.

The cytoplasmic ribosome participates in the dorsoventral patterning of Arabidopsis leaves, as shown by the hypomorphic or null alleles of several genes encoding ribosomal

proteins, which increase the severity of the ventralization caused by loss of function of ASYMMETRIC LEAVES1 (AS1) and AS2; the AS1-AS2 repressor complex is essential for dorsal identity specification in leaf tissues. The *sca1 as2* double mutants exhibited a synergistic phenotype. We demonstrated that *SCA1* is At2g33800, whose product is RPS5, a component of the small subunit of the chloroplast ribosome. This is an unexpected discovery, since it indicates that not only the cytoplasmic ribosome, but also the chloroplast ribosome, is involved in dorsoventral pattern formation. On the other hand, *SCA5* is At5g20040, which encodes TRNA ISOPENTENYL TRANSFERASE 9 (IPT9), involved in the biosynthesis of cis-zeatin, a cytokinin.

Leaf mutants are not only useful for identifying genes specifically involved in leaf organogenesis but may also provide information on the developmental mechanisms that leaves share with other plant organs. This has been the case for *icu11-1*. Previous analyses performed in the laboratory of J.L. Micol concluded that *icu11-1* (a) genetically interacts with alleles of genes encoding components of the epigenetic machinery, such as *ICU1* (also known as *CURLY LEAF*; *CLF*) and *ICU2*, (b) causes the ectopic and heterochronic derepression of flower organ identity genes in leaves, and (c) is an allele of At1g22950, which encodes a putative 2-oxoglutarate and Fe^{2+}-dependent dioxygenase (2OGD).

Here, we stablished that *ICU11* belongs to a five-member gene family of the 2OGD superfamily, which we named CUPULIFORMIS (CP). *ICU11* and its closest paralog *CP2* are functionally redundant: the *icu11 cp2* double mutants skip vegetative development and flower immediately after germination. This lethal phenotype is reminiscent to that caused by loss-of-function alleles of *EMBRYONIC FLOWER 1* (*EMF1*) and *EMF2*, which encode Polycomb group proteins with known epigenetic functions. Massive sequencing of *icu11-1* RNA revealed the deregulation of hundreds of genes, some of which regulate flower development, such as those of the MADS-box family. Derepression of one of these genes, *SEPALLATA3* (*SEP3*), causes leaf hyponasty in *icu11-1* plants. Massive bisulfite sequencing and chromatin immunoprecipitation of *icu11-1* demonstrated that *ICU11* and *CP2* participate in histone chemical modification but not in DNA methylation. The most important result of this Thesis is the identification of a family of epigenetic machinery components whose activity seems different to those previously described.